added by way of these amendments.

Amendments to Correct Typographical Errors Submitted March 10, 2003

By way of the previous Amendment submitted to the USPTO on March 10, 2003, Applicant amended the specification merely to correct inadvertent typographical and editing errors, and to correctly identify the Sequence Identification numbers. In the Office Action mailed on June 2, 2003 (Paper No. 10), the Examiner objected to the amendments to correct typographical errors as allegedly being new matter. Applicant respectfully contends and submits that the amendments do not constitute new matter, but rather, merely correct inadvertent typographical and editing errors, for the following reasons.

In a telephone conversation between the Examiner and Applicant's representative, Thomas Sossong, on June 23, 2003, Dr. Sossong explained to the Examiner that the amendments submitted by way of the previous Amendment on March 10, 2003, were identical to amendments submitted by way of a Preliminary Amendment in each of several parent patent applications, of which the present application is a Continuation application. As indicated in the Preliminary Amendment filed along with the present application on December 3, 2001, the present application is a continuation of U.S. application no. 09/333,412, now U.S. Patent 6,342,382, which is a continuation of U.S. application no. 08/878,360, now U.S. Patent no. 5,945,322, which is a continuation of application no. 08/683,426, now U.S. Patent no. 5,705,367, which is a division of application no. 08/312,387, now U.S. Patent no. 5,545,553.

In light of Dr. Sossong's explanation of the reason for the March 10, 2003 amendment to the specification, the Examiner kindly indicated that submission of evidence regarding the nature of similar amendments made by way of a Preliminary Amendment in parent applications, in addition to submission of evidence supporting such amendments in the issued patents in the application family, as set forth in the paragraph above, would help the Examiner in reconsidering the "new matter" objection.

Applicant thanks the Examiner for the courtesy shown his representative during the telephone call, and submits the requested information as described more fully below. The amendments to the specification made in the March 10, 2003 Amendment,

and again described in detail herein below correspond to amendments made in each of the above-referenced related patent applications for the purpose of correcting the same inadvertent typographical and editing errors, and accordingly, such amendments merely serve to ensure that the present application is in conformance with the applications to which it claims priority.

Summary of Amendments to the Specification in Amendment Submitted March 10, 2003

Applicant amended the specification by way of the Amendment submitted to the USPTO on March 10, 2003, merely to correct inadvertent typographical and editing errors regarding the Sequence Identification numbers, and to correctly identify the Sequence Identification numbers, and to bring the instant application into conformance with parent patent applications that have now issued as U.S. Patents.

Summary of Amendments to Claims 34 and 39 in Amendment Submitted March 10, 2003

In the March 10, 2003 Amendment, claims 34 and 39 were amended to correctly refer to the Sequence Identification numbers.

The present application claims, in part, an invention comprising five (5) novel glycosyltransferases from *Neisseria*. Specifically, the present application claims lgtA, lgtB, lgtC, lgtD, and lgtE polypeptides.

Accordingly, the five glycosyltransferases of the present invention are the object of claims 34-39, as indicated by the recitation of the identifying term, "glycosyltransferase," followed by the recitation of a SEQ ID number for each one of the LgtA, LgtB, LgtC, LgtD, and LgtE glycosyltransferases. SEQ ID NO:3 correctly refers to lgtA, SEQ ID NO:4 correctly refers to lgtC, SEQ ID NO:5 correctly refers to lgtD, and SEQ ID NO:6 correctly refers to lgtE. However one of the five glycosyltransferases of the present invention, LgtB, was incorrectly referred to using the term "SEQ ID NO:2" in claims 34 and 39.

SEQ ID NO:2, as set forth in the Sequence Listing in the present application, is a polynucleotide sequence encoding a glycyl tRNA synthetase beta chain, and is not a *Neisseria* glycosyltransferase. SEQ ID NO:8 correctly refers to LgtB, a

glycosyltransferase of the present invention. Therefore, the above-described amendments to the application served in part to make proper reference to the LgtB glycosyltransferase (SEQ ID NO:8) of the present invention, and to eliminate any incorrect reference to glycyl tRNA synthetase (SEQ ID NO:2), which is not claimed as part of the present invention.

More particularly, claims 34 and 39 were amended in part to correctly refer to the Sequence Identification numbers, which numbers were amended by way of the March 10, 2003 Amendment to correct inadvertent typographical and editing errors. Specifically, the incorrect recitation of "SEQ ID NO:2" in claims 34 and 39 was changed so that the claims now correctly recited "SEQ ID NO:8." Prior to the March 10, 2003 amendment, claims 34 and 39 incorrectly recited, "...glycosyltransferase comprising the amino acid sequence SEQ ID NO:2, ..." Amended claims 34 and 39 properly recite "SEQ ID NO:8," which is the LgtB glycosyltransferase of the present invention.

All of the above-detailed amendments to the present application were merely made to correct informalities with the application. Accordingly, Applicant respectfully submits that no new matter has been added by way of the amendments of March 10, 2003. Support for all of the above-described amendments to the application can be found in the as-filed application, and specifically, in the Sequence Listing.

The Sequence Listing filed in connection with the present application sets forth that SEQ ID NO:3 is LgtA, SEQ ID NO:4 is LgtC, SEQ ID NO:5 is LgtD, SEQ ID NO:6 is LgtE, and that SEQ ID NO:8 is LgtB. Specifically, SEQ ID NO:1 sets forth a *Neisseria* polynucleotide sequence which also displays the amino acid translation of the coding regions of SEQ ID NO:1. The "Information for SEQ ID NO:1" set forth on pages 1 and 2 of the Sequence Listing of the present application defines SEQ ID NO:1 as encoding glycyl tRNA synthetase beta chain from nucleotides 1-381 of SEQ ID NO:1, encoding LgtA from nucleotides 445-1491 of SEQ ID NO:1, encoding LgtC from nucleotides 2342-3262 of SEQ ID NO:1, encoding LgtD from nucleotides 3322-4335 of SEQ ID NO:1, and encoding LgtE from nucleotides 4354-5196 of SEQ ID NO:1.

Each of the five proteins encoded by SEQ ID NO:1 – four glycosyltransferases and one glycyl tRNA synthetase – are individually set forth in SEQ ID NOS:2-6 in the Sequence Listing. SEQ ID NO:2 sets forth the amino acid sequence

for the glycyl tRNA synthetase beta chain encoded by nucleotides 1-381 of SEQ ID NO:1, SEQ ID NO:3 sets forth the amino acid sequence for the LgtA glycosyltransferase encoded by nucleotides 445-1491 of SEQ ID NO:1, SEQ ID NO:4 sets forth the amino acid sequence for the LgtC glycosyltransferase encoded by nucleotides 2342-3262 of SEQ ID NO:1, SEQ ID NO:5 sets forth the amino acid sequence for the LgtD glycosyltransferase encoded by nucleotides 3322-4335 of SEQ ID NO:1, and SEQ ID NO:6 sets forth the amino acid sequence for the LgtE glycosyltransferase encoded by nucleotides 4354-5196 of SEQ ID NO:1.

Also in the Sequence Listing filed in connection with the present application, SEQ ID NO:7 sets forth a *Neisseria* polynucleotide sequence which also displays the amino acid translation of the coding regions of SEQ ID NO:7. The "Information for SEQ ID NO:7" set forth on page 14 of the Sequence Listing of the present application defines SEQ ID NO:7 as encoding LgtB from nucleotides 1491-2330 of SEQ ID NO:7. SEQ ID NO:8 individually sets forth the amino acid sequence for the LgtB glycosyltransferase encoded by nucleotides 1491-2330 of SEQ ID NO:7.

Evidence Supporting Similar Amendments to the Specification in Parent Applications

Applicant directs the Examiner's attention to three Exhibits submitted herewith:

Exhibit A = Preliminary Amendment submitted at the time of filing of parent patent application no. 08/878,360, now U.S. Patent no. 5,945,322

Exhibit B = Copy of the "Marked-up version to show changes made" from the Amendment submitted to the USPTO on March 10, 2003 in connection with the instant application

Exhibit C = U.S. Patent no. 5,945,322

Applicants respectfully submit that the amendments to the specification described above were identically made in the parent applications resulting in U.S. Patents No. 5,945,322, 5,705,367, and 5,545,553. However, for the sake of clarity, Applicant will use only one of the parent patents – U.S. Patent no. 5,945,322 – as evidence of amendments identical to those submitted by way of the Amendment filed with the

USPTO on March 10, 2003.

For example, Applicant directs the Examiner's attention to page 1 of Exhibit A to the third and fourth paragraphs beginning with the phrase "On page..., " as indicated by the arrows marked "A1" and "A2, " respectively. In comparison, Applicant directs the Examiner's attention to page 21 of Exhibit B, and in particular, to the marked-up replacement paragraphs directing insertion "at lines 15-17 of page 7" and "at lines 1-13 on page 8," as indicated by the arrows marked "B1" and "B2, " respectively. Applicant respectfully submits that the amendment at A1 inserting the term "SEQ ID NO:8" and deleting the term "SEQ ID NO:2" in the parent application is identical to the amendment at B1 inserting the term "SEQ ID NO:8" and deleting the term "SEQ ID NO:2" in the instant application. Similarly, the amendment at A2 inserting the term "SEQ ID NO:8" and deleting the term "SEQ ID NO:2" in the parent application is identical to the amendment at B2 inserting the term "SEQ ID NO:8" and deleting the term "SEQ ID NO:2" in the instant application. Accordingly, Applicant respectfully submits that the amendment to the instant application, submitted on March 10, 2003, does not add new matter to the application, as identical amendments to correct typographical errors were made in the parent applications.

Further support for the above-described amendments in the application that issued as parent U.S. Patent No. 5,945,322 ("the '322 patent") can be found in the published patent itself, submitted herewith as Exhibit C. Specifically, Applicant directs the Examiner's attention to the term "SEQ ID NO:8" in line 65 of column 4 in Exhibit C. This term corresponds to the amendments indicated above at location A1 of Exhibit A and location B1 of Exhibit B. Similarly, Applicant directs the Examiner's attention to the term "SEQ ID NO:8" in line 13 of column 5 in Exhibit C. This term corresponds to the amendments indicated above at location A2 of Exhibit A and location B2 of Exhibit B. Accordingly, applicant respectfully submits that the amendments to the instant application, submitted on March 10, 2003, do not add new matter to the application, as identical amendments to correct typographical errors made in the parent applications are present in the published patents issuing from the parent applications.

Applicant also directs the Examiner's attention to amendments on pages 2 and 3 of Exhibit A and respectfully submits that such amendments submitted in the

parent application are identical to those amendments found on pages 21-28 of Exhibit B, which amendments were submitted by way of Amendment on March 10, 2003, in connection with the instant application. Applicant further submits that evidence of all amendments made in the parent application can be found in the issued patent, Exhibit C. Further, Applicant directs the Examiner's attention to the first paragraph under the "REMARKS" section in Exhibit A, which paragraph sets forth that the amendments were made to correct inadvertent typographical and editing errors, and to identify the correct SEQ ID NOs.

Applicant respectfully submits that the above-detailed amendments, as well as the entirety of the amendments to the specification of the instant application as submitted by way of a Preliminary Amendment on March 10, 2003, do not add new matter to the application. Accordingly, Applicants respectfully submit that the Examiner's objection to the March 10, 2003 amendments to the specification as constituting "new matter" is improper and should be withdrawn. Applicant kindly invites the Examiner to discuss this matter with Applicant's representatives should the Examiner require further clarification or explanation.

Rejection under 35 U.S.C. § 112, first paragraph

The Examiner has rejected claims 34-49 under 35 U.S.C. § 112, first paragraph, as allegedly lacking adequate written description in the specification. Specifically, it is the Examiner's view that the application does not indicate that Applicant was in possession of all the polypeptide sequences encompassed by the claims. Applicant has canceled claims 40-49, rendering the Examiner's rejection of those claims moot. Insofar as the Examiner's rejection pertains to remaining claims 34-39 and newly added claims 50-58, Applicant respectfully traverses the rejection for the reasons set forth below.

Applicant has amended claims 34-39 herein to remove language directed to "functionally active fragments" of the claimed polypeptides. For example, claim 34 now reads, "An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:8." Applicant respectfully submits that the polypeptides encompassed by claims 34-39 are set forth in the corresponding SEQ ID NOs in the instant application, and that

claims 34-39, as amended, fully satisfy the written description requirement of 34-49 under 35 U.S.C. § 112, first paragraph. Further, newly added claims 50-58 depend from claims 34-39, and therefore similarly satisfy the written description requirement. Accordingly, Applicant respectfully submits that the rejection of claims 34-39 is improper and should be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 34-49 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out and distinctly claim the subject matter that the applicant regards as the invention. Specifically, it is the Examiner's view that the metes and bounds of the phrase "functionally active fragment thereof" is not clear. Applicant respectfully traverses this rejection. As described above, applicant has canceled claims 40-49, rendering the Examiner's rejection of those claims moot. Insofar as the Examiner's rejection pertains to remaining claims 34-39 and newly added claims 50-58, Applicant respectfully traverses the rejection for the reasons set forth below.

Claims 34-39 have been amended herein to remove language directed to "functionally active fragments" of the claimed polypeptides, as set forth in detail above in response to the rejection under 35 U.S.C. § 112, first paragraph. Applicant respectfully submits that the polypeptides encompassed by claims 34-39 are set forth in the corresponding SEQ ID NOs in the instant application, and that claims 34-39, as amended, fully satisfy the written description requirement of 35 U.S.C. § 112, first paragraph. Further, newly added claims 50-58 depend from claims 34-39, and therefore similarly satisfy the written description requirement. Accordingly, Applicant respectfully submits that the rejection of claims 34-39 is improper and should be withdrawn.

The Examiner has also rejected claims 34-49 under 35 U.S.C. § 112, second paragraph, because in the Examiner's view, the phrase "a glycosyltransferase" does not particularly point out and distinctly claim the subject matter Applicant regards as the invention. The Examiner further states that the term "glycosyltransferase" is a very broad or general name for an enzyme. Applicant respectfully contends that the "glycosyltransferase" of each claim is indeed particularly identified and distinctly

claimed by the inclusion of a specific and unique SEQ ID NO in each of independent claims 34-39.

Applicant respectfully submits that the term "glycosyltransferase" is well-known in the art, and is also defined in numerous places in the instant specification. For Example, on page 27 of the specification, lines 7-10 describe that glycosyltransferases of the invention are useful in the biosynthesis of oligosaccharides, and that glycosyltransferases of the invention "are capable of stereospecific conjugation of a specific activated saccharide unit to a specific acceptor molecule." Therefore, the specificity of a glycosyltransferase is known to one of skill in the art by the donor molecule specificity and acceptor molecule specificity of the enzyme. Applicants have set forth such details clearly in the instant specification. For example, the paragraph at lines 10-20 on page 46 of the instant specification describes the acceptor and donor specificities of the glycosyltransferases of the present invention. Specifically, lgtA (SEQ ID NO:3) transfers a GalNAc donor $\beta 1 \rightarrow 3$ to a Gal acceptor, lgtB (SEQ ID NO:8) transfers a Gal donor $\beta 1 \rightarrow 4$ to a GlcNAc acceptor, lgtC (SEQ ID NO:4) transfers a Gal donor $\alpha 1 \rightarrow 4$ to a Gal acceptor, lgtD (SEQ ID NO:5) transfers a GlcNAc donor $\beta 1 \rightarrow 3$ to a Gal acceptor, and lgtE (SEQ ID NO:6) transfers a Gal donor $\beta 1 \rightarrow 4$ donor to a Glc acceptor. Each of the five glycosyltransferases of the invention is further and specifically defined by it's polypeptide sequence, as set forth immediately above and particularly in each of the pending claims of the present application. Accordingly, Applicant respectfully submits that the term "a glycosyltransferase" as used in the claims is not indefinite and respectfully requests reconsideration and withdrawal of the rejection.

The Examiner has also rejected claims 40-49 under 35 U.S.C. § 112, second paragraph, because in the Examiner's view, all of claims 40-49 are drawn to the "same method involving the transfer of the same carbohydrate groups to the very same acceptor moieties but in the presence of different polypeptides." While not necessarily agreeing with the Examiner's reasoning, Applicant has cancelled claims 40-49 herein. Accordingly, Applicant respectfully submits that the rejection has been rendered moot and requests withdrawal of the rejection.

Insofar as the Examiner's rejection applies to newly added claims 50-58,

Applicant respectfully disagrees for the following reason. New claims 50-58 claim the transfer of distinct carbohydrate groups to distinct acceptor moieties, depending upon the identity of the particular *Neisseria* lgt glycosyltransferase specified in each claim. Accordingly, Applicants respectfully submit that the Examiner's rejection would not apply to new claims 50-58.

Summary

The amendments made herein are supported in the as-filed specification, and as such, no new matter has been added by way of the present amendment. Applicant respectfully submits that each and every rejection or objection set forth by the Examiner has either been overcome or is now inapplicable, and that the instant application is in full condition for allowance. Favorable examination of the claims on the merits is respectfully requested.

Respectfully submitted,
EMIL C. GOTSCHLICH

August 27, 2003 By: Kathryn Doyle
(Date)

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Enclosures (Exhibts A, B & C)

EXHIBIT A

Preliminary Amendment submitted at the time of filing of parent patent application no. 08/878,360, now U.S. Patent no. 5,945,322

Submitted in conjunction with the Amendment on (Date:)_____
responsive to the Office Action dated June 2, 2003 (Paper No. 10), sent in connection with U.S. Patent Application Number 10/007,267

Express Mail _____

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Emil Gotschlich

Serial No.: To be assigned — Group Art Unit: To be assigned

Filed: Herewith — Examiner: To be assigned

For: GLYCOSYLTRANSFERASES FOR BIOSYNTHESIS OF OLIGOSACCHARIDES, AND GENES ENCODING THEM — Attorney Docket No.: 7188-031

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with Rule 115 of the Rules of Practice, please consider the following amendments and remarks.

IN THE SPECIFICATION

Please amend the specification as follows:

On page 4, line 15, after "synthesis" delete "of".

On page 6, line 19, after "shown in" delete "Figure 2"; and line 22, after "shown in" delete "Figure 2".

On page 7, line 1, after "shown in" delete "Figure 2"; line 5, "shown in" delete "Figure 2"; line 16, after "sequence of" delete "SEQ ID NO:2" and after "SEQ ID NO:3" insert --or SEQ ID NO:11--; and on line 17, after "SEQ ID NO:5 or," insert --SEQ ID NO:12--, and after "NO:6" insert --, or SEQ ID NO:8--.

A1

On page 8, line 2, delete "SEQ ID NO:2" after "SEQ ID NO:3" insert --or SEQ ID NO:11 --; on line 3, after "NO:5" delete ", or " and insert --or SEQ ID NO:12--, and after "NO:6," insert --or SEQ ID NO:8,--; on line 7, delete "SEQ ID NO:2" and insert

A2

therefor -- SEQ ID NO:3 or SEQ ID NO:11 --; on line 9, delete "NO:3" and insert therefor -- NO:8--; on line 11, after "SEQ ID NO:5" insert -- or SEQ ID NO:12 --; on line 20, delete "NO:2" and insert therefor -- NO:3 or SEQ ID NO:11 --; on line 23, delete "NO:3" and insert therefor -- NO:8--; and on line 30, after "SEQ ID NO:5" insert -- SEQ ID NO:12 -- .

On page 11, line 3, after "Figure 2:" delete "(A)"; line 10-13, delete "(B) Amino acid sequences of LgtA (SEQ ID NO:2), LgtB (SEQ ID NO:3), LgtC (SEQ ID NO:4), LgtD (SEQ ID NO:5), and LgtE (SEQ ID NO:6), and the nucleotide sequence of the Igt locus (SEQ ID NO:1)."; line 13, after "Figure 3" insert -- (A and B)--, after "*lgtA*" insert --(SEQ ID NO:11)-- and after "IgtD" insert --(SEQ ID NO:12)--; line 19, after "Figure 4" insert -- (A and B) --: and line 24, after "Figure 5" insert -- (A and B) --.

On page 15, line 1, delete "SEQ ID NO:2,"; and on line 17, delete "or SEQ ID NO:6" and insert therefor -- SEQ ID NO:6, or SEQ ID NO:8 --.

On page 30, line 20, delete "NO:2" and insert therefor -- NO:3 or SEQ ID NO:11 --; line 24, delete "NO:3" and insert therefor -- NO:8 --; and line 29, after "SEQ ID NO:5" insert --or SEQ ID NO:12 --.

On page 31, line 10, delete "NO:2 and insert therefor -- NO:3 or SEQ ID NO:11 --; and line 14, delete "NO:3" and insert therefor -- NO:8 --.

On page 38, line 13, delete "random-prime-labeled" and insert therefor -- random-primer-labeled --; and line 25, after "presented in" delete "Figure 2" and insert therefor -- SEQ ID NO: 1 --.

On page 39, line 20, delete "NO:7" and insert therefor --NO:9--, and delete NO:8" and insert therefor -- NO:10 --.

On page 41, line 27, delete 258:10637 and insert therefor -- 258:10637)--

On page 42, line 2, delete "Ifound" and insert therefor -- were found --; and line 22, delete "rbs" and insert therefor -- rbs, --; and line 22, delete "*IgtD*" and insert therefor -- ltd, --.

Please insert the enclosed SEQUENCE LISTING after page 49 and before the claims.

IN THE DRAWINGS

Please amend Figure 2 to cancel Figure 2B and renumber Figure 2A as Figure 2.

IN THE CLAIMS

Please amend the claims as follows:

In claim 1, line 3, delete "Figure 2 (SEQ ID NO:1)" and insert therefor -- SEQ ID NO:1 --.

In claim 2, lines 3-4, delete "Figure 2 (SEQ ID NO:1)" and insert therefor -- SEQ ID NO:1 --.

In claim 4, lines 2-3, delete "Figure 2 (SEQ ID NO:1)" and insert therefor -- SEQ ID NO:1 --.

In claim 6, lines 2-3, delete "Figure 2 (SEQ ID NO:1)" and insert therefor -- SEQ ID NO:1 --.

REMARKS

The specification has been amended to correct inadvertent typographical and editing errors, and to identify the correct Sequence Identification numbers. The specification has additionally been amended to incorporate two additional sequences: SEQ ID NO:11 and SEQ ID NO:12. These sequences correspond to lgtA (SEQ ID NO:3) and lgtD (SEQ ID NO:5), with the exception that the first amino acid is leucine (LEU) rather than methionine (MET). Indeed, the codon in SEQ ID NO:1 at this position (TTG) ordinarily encodes leucine; however, in *Neisseria*, a TTG start codon encodes methionine.

Thus, the sequences of SEQ ID NO:3 and SEQ ID NO:5 are correct. However, the amino acid sequences for lgtA and lgtD in Figure 3 include leucine as the first amino acid, *i.e.*, as would be found if the protein were expressed in a non-*Neisseria* expression system, such as, those expression systems described in the specification at page 25, lines 1-12.

Figure 2 has been amended to delete Figure 2B, which corresponds to the SEQUENCE LISTING.

Claims 1-15 are pending in the application. Claims 16-33 have been canceled. Claims 1, 2, 4, 6 have been amended, in order to more particularly point out and distinctively claim that which applicants regard as their invention. In light of the deletion of Figure 2B, references in the claims to the sequence information in Figure 2B have been corrected to refer to the appropriate Sequence Identification Number. Support for the amendment to the claims is found throughout the application as filed.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks in the file history of the instant Application. The claims as amended are believed to be in condition for allowance. Early favorable action on the claims is earnestly solicited.

Respectfully submitted,

Date_____

26,742
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EXHIBIT B

Copy of the "Marked-up version to show changes made" from the Amendment submitted to the USPTO on March 10, 2003 in connection with the instant application

Submitted in conjunction with the Amendment on (Date:) _____
responsive to the Office Action dated June 2, 2003 (Paper No. 10), sent in connection with U.S. Patent Application Number 10/007,267

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO: ASSISTANT COMMISSIONER OF PATENTS, WASHINGTON, DC 20231, ON THE DATE INDICATED BELOW.
BY: Judy Kawoshi DATE: March 10, 2003

BOX PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Patent Application of Emil C. Gotschlich	: Group Art Unit: 1652
Appln. No.:	10/007,267	: Examiner: Manjunath Rao
Filed:	December 3, 2001	:
For:	GLYCOSYLTRANSFERASES FOR BIOSYNTHESIS OF OLIGOSACCHARIDES AND GENES ENCODING THEM	: Attorney Docket No. **040853-01-5029-02**

AMENDMENT

This Amendment responds to the Office Action dated December 9, 2002 (Paper No. 6), sent in connection with the above-identified application. This Amendment is being timely filed in view of the shortened statutory period for reply, which sets the time for a response to the Office Action to and through March 10, 2003 (March 9, 2003 being a Sunday).

In response to the Office Action, kindly amend the application as follows:

In the Specification:

Please delete the paragraph from page 4 line 15 to page 5 line 2, and insert the following paragraph in place thereof:

-- Little information on the genetics of LOS synthesis in Neisseria is available. A major advance has been the creation (Dudas and Apicella, 1988, Infect. Immun. 56:499) and biochemical characterization (John et al., 1991, J. Biol. Chem. 266:19303) of five pyocin mutants of gonococcal strain 1291, dubbed 1291a-e. Immunological and biochemical data have shown that 1291a, 1291c, 1291d and 1291e produce LOS with sequential shortening of the lacto-N-neotetraose chain, with mutant 1291e lacking the glucose substitution on the heptose. Mutant 1291b synthesizes the

alternative LOS structure Galα1 $\rightarrow$ 4Galβ1 $\rightarrow$ 4Glc (see Figure 1). Only the genetic basis of the 1291e mutant is now defined. It is a mutation of phosphoglucomutase (pgm), which precludes the synthesis of UDP-glucose, and hence the addition of the first residue of the lacto-N-neotetraose unit (Zhou et al., 1994, J. Biol. Chem. 269:11162; Sandlin and Stein, 1994, J. Bacteriol. 176:2930). It also has been shown that gale mutants of meningococcus or gonococcus produce truncated LOS in keeping with the inability to synthesize UDP-galactose (Robertson et al., 1993, Molec. Microbiol. 8:891; Jennings et al., 1993, Molec. Microbiol. 10:361). --

Please delete the paragraph at lines 13-23 on page 6 and insert the following paragraph in place thereof:

-- The present invention is directed to nucleic acids encoding glycosyltransferases, the proteins encoded thereby, and to methods for synthesizing oligosaccharides using the glycosyltransferases of the invention. Accordingly, in one aspect, the invention is directed to a purified nucleic acid that is hybridizable under moderately stringent conditions to a nucleic acid corresponding to the LOS locus of Neisseria, e.g., a nucleic acid having a nucleotide sequence corresponding to or complementary to the nucleotide sequence shown in (SEQ ID NO:1). Preferably, the nucleic acid of the invention is hybridizable to a portion of the coding sequence for a gene of the LOS locus, i.e., a portion of the nucleotide sequence shown in (SEQ ID NO:1) that encodes a functionally active glycosyltransferase. --

Please delete the paragraph on page 6 line 24 to page 7 line 5 and insert the following paragraph in place thereof:

-- In specific embodiments, the invention relates to a nucleic acid that has a nucleotide sequence corresponding to or complementary to a portion of the nucleotide sequence shown in (SEQ ID NO:1) that encodes a functionally active glycosyltransferase. In a further aspect, the nucleic acid encodes a functionally active glycosyltransferase. In a specific embodiment, the invention is directed to a nucleic acid that has a nucleotide sequence corresponding to or complementary to the nucleotide sequence shown in (SEQ ID NO: 1). --

Please delete the paragraph at lines 15-17 on page 7 and insert the following paragraph in place thereof:

-- In specific embodiments, exemplified herein, the nucleic acid encodes a glycosyltransferase having an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:11, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:12, SEQ ID NO:6, or SEQ ID NO:8. --

Please delete the paragraph at lines 1-13 on page 8 and insert the following paragraph in place thereof:

-- In a primary aspect, the invention is directed to glycosyltransferase having an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:11, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:12, SEQ ID NO:6, or SEQ ID NO:8 or a functionally active fragment thereof. The invention further contemplates a composition comprising a glycosyltransferase conjugated to a solid phase support, wherein the glycosyltransferase is selected from the group consisting of a glycosyltransferase having an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:11, or a functionally active fragment thereof; a glycosyltransferase having an amino acid sequence of SEQ ID NO:8, or a functionally active fragment thereof; a glycosyltransferase having an amino acid sequence of SEQ ID NO:4, or a functionally active fragment thereof; and a glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12, or a functionally active fragment thereof; and a glycosyltransferase having an amino acid sequence of SEQ ID NO:6, or a functionally active fragment thereof. --

Please delete the paragraph from page 8 line 14 to page 9 line 3, and insert the following paragraph in place thereof:

-- Having provided novel glycosyltransferases, and genes encoding the same, the invention accordingly further provides methods for preparing oligosaccharides, *e.g.*, two or more saccharides. In specific embodiments, the invention relates to a method for adding GalNAc or GlcNAcβ1→3 to Gal, comprising contacting a reaction mixture comprising an activated GalNAc or GlcNAc to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ

ID NO:3 or SEQ ID NO:11; a method for adding Galβ1→4 to GlcNAc or Glc, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a GlcNAc or Glc residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:8; a method for adding Gal α1→4 to Gal, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:4; a method for adding GalNAc or GlcNAc β1→3 to Gal, comprising contacting a reaction mixture comprising an activated GalNAc or GlcNAc to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12; and a method for adding Gal β1→4 to GlcNAc or Glc, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a GlcNAc or Glc residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:6. --

Please delete the paragraph from lines 3-12 on page 11, and insert the following paragraph in place thereof:

-- Figure 2: Genetic map of the LOS locus based on the DNA sequence. Sequence information bp 1-2725 was obtained from plasmid pPstCla, bp 2725-5859 from plasmid p3400 (see materials and methods). IS refers to an area of the sequence that has homology to a previously reported neisserial insertion sequence IS1106 (Knight et al., 1992, Molec. Microbiol. 6:1565). The positions of the reading frames of *lgtA-E* are indicated. Three tracts of poly-G were found in *lgtA* (17 bp), *lgtC* (10 bp) and *lgtD* (11 bp) and are indicated by vertical black bars. --

Please delete the paragraph from lines 13-18 on page 11, and insert the following paragraph in place thereof:

-- Figure 3 (A and B): Homology of the protein products of *lgtA* (SEQ ID NO:11) and *lgtD* (SEQ ID NO:12). The primary structure of two proteins is very similar, particularly in the first half of the sequences. The glycine residues starting at position 86

reflect the coding of the poly-G regions in the respective genes. The Bestfit program of the GCG package was used and the symbols |, :, . represent degrees of similarity based on the Dayhoff PAM-250 matrix. --

Please delete the paragraph from lines 19-23 on page 11, and insert the following paragraph in place thereof:

-- Figure 4 (A and B): Homology of the protein products of *lgtB* (SEQ ID NO:8) and *lgtE* (SEQ ID NO:6). The primary 20 structure of two proteins is very similar, particularly in the first half of the sequences. These sequences also have significant homology to *lex-1* (Cope et al., i99i, Molec. Microbiol. 5:i113) or *lic2A* (High et al., i993, Molec. Microbiol. 9:1275) genes of *Haemophilus influenzae*. For meaning of symbols see Figure 3. --

Please delete the paragraph from page 11 line 24 to page 12 line 2, and insert the following paragraph in place thereof:

-- Figure 5 (A and B): Homology of the protein products of *rfaI* (SEQ ID NO:13) and *lgtC* (SEQ ID NO:4). The *E. coli rfaI* and *rfaJ* genes are very closely related. They serve as glucosyl transferases of two glucose residues in the LPS core region (Pradel et al., 1992, J. Bacteriol. 174:4736). The glycines at position 54-56 in *lgtC* are encoded by the poly-G tract. For meaning of symbols see Figure 3. --

Please delete the paragraph from lines 11-29 on page 30, and insert the following paragraph in place thereof:

-- Accordingly, a method for preparing an oligosaccharide having the structure GalNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc *(i.e.,* ganglioside) comprises sequentially performing the steps of:

a. contacting a reaction mixture comprising an activated Gal to an 15 acceptor moiety comprising a Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO: 6, or a functionally active fragment thereof;

b. contacting a reaction mixture comprising an activated GlcNAc to the acceptor moiety comprising a Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:11, or a functionally active fragment thereof;

c. contacting a reaction mixture comprising an activated Gal to the acceptor moiety comprising a GlcNAcβ1→3Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid of SEQ ID NO:8; and

d. contacting a reaction mixture comprising an activated GalNAc to the acceptor moiety comprising a Galβ1→4GlcNAcβ1→3Ga1β1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12, or a functionally active fragment thereof. --

Please delete the paragraph from lines 1-14 on page 31, and insert the following paragraph in place thereof:

-- Similarly, a method for preparing an oligosaccharide having the structure Galβ1→4GlcNAcβ1→3Ga1β1→4Glc *(i.e.,* lacto-N-neotetraose) comprises sequentially performing the steps of:

a. contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO: 6, or a functionally active fragment thereof;

b. contacting a reaction mixture comprising an activated GlcNAc to the acceptor moiety comprising a Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:11, or a functionally active fragment thereof; and

c. contacting a reaction mixture comprising an activated Gal to the acceptor moiety comprising a G1cNAcβ1→3Ga1β1→4Glc residue in the presence of a glycosyltransferase having an amino acid of SEQ ID NO:8. --

Please delete the paragraph from lines 10-26 on page 38, and insert the following paragraph in place thereof:

-- A gene bank of *Neisseria gonorrhoeae* strain F62 genomic DNA was constructed by ligating ca 20 kb fragments obtained by incomplete digestion with *Sau3A* into *Bam*HI/*Eco*RI digested λ2001 (Karn et al., 1984, Gene 32:217). The phage library was screened by hybridization with random-primer-labeledplasmid pR10PI, and 5 clones were isolated by plaque purification. The phage from these clones were purified by sedimentation followed by flotation on CsCl (Davis et al., 1980, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), and the DNA was isolated. From one of these clones, two *Cla*I fragments of 4.9 and 3.4 kb were isolated by gel electrophoresis and recovery with Geneclean II (BIO 101 Inc., La Jolla, CA). These were ligated into *Cla*I cut pBluescript II SK- from Stratagene (La Jolla, CA) and called p4900 and p3400 respectively. p4900 contained a *Pst*I site in the insert and was subdivided into two clones containing inserts of 2.1 and 2.8 kb. The clone containing the 2.8 kb insert was called pPstCla. The inserts in p3400 and pPstCla were sequenced by the chain termination method (Sanger et al., 1977, Proc. Natl. Acad Sd. USA 74:5463) using Sequenase II, (United States Biochemical Co., Cleveland, OH). All of the sequence presented in SEQ ID NO:1 was completed in both directions. --

Please delete the paragraph from line 13 page 39 to line 3 page 40, and insert the following paragraph in place thereof:

-- Transformation of piliated *Neisseria gonorrhoeae* strain F62 was performed with plasmids isolated from *E. coil* (Klugman et al., 1989, Infect. Immun. 57:2066) and the transformants selected on GC agar (Swanson, 1978, Infect. Immun. 19:320) containing 2 μg/ml erythromycin. The fidelity of the genomic alteration of each of the gonococcal transformants was verified by sequencing the upstream and downstream junctions of the *ermC'* gene in their genomic DNA using a PCR technique. Two 5' biotinylated primers, GCCGAGAAAACTATTGGTGGA (SEQ. ID. NO:9) and AAAACATGCAGGAATTGACGAT) (SEQ. ID. NO:10), were synthesized; these were based on the *ermC'* sequence near its upstream and its downstream end respectively. The primers were designed such that their 3' ends pointed outward from the *ermC'* gene.

Each of these primers was used together with a suitable primer matching the sequence of the LOS locus near the putative insertion. PCR was performed according the instructions supplied with the GeneAmp PCR Reagent Kit from Perkin Elmer (Branchburg, NJ) using 25 cycles. In all instances the expected size product was obtained. The DNA sequence of these products was determined by purifying the PCR product on magnetic streptavidin beads from Dynal, Inc. (Lake Success, NY) and sequencing with the Sequenase II kit according to a protocol provided by Dynal, Inc., based on the method developed by Hultman et al (Hultman et al., 1989, Nucleic Acids Res. 17:4937). The sequences were analyzed by computer programs in the GCG package of Genetics Computer Group, Inc. (Madison, WI). --

Please delete the paragraph from lines 15-29 on page 41, and insert the following paragraph in place thereof:

-- A λ2001 bank of *Neisseria gonorrhoeae* strain F62 DNA was screened by hybridization with pR10PI and 5 clones were isolated. One of these clones, when digested with either *Cla*I or *Bfa*I and examined by Southern hybridization using pR10PI as the probe, gave rise to a pattern identical to that seen with genomic DNA. The appropriate *Cla*I fragments of this λ2001 clone were isolated and cloned into the *Cla*I site of pBluescript II SK-. The entire sequence of the 3400 *Cla*I fragment was determined. Mapping of the clone containing the 4900 bp *Cla*I fragment indicated that there was a single *Pst*I site in the clone about 2.8 kb from one side, allowing the clone to be divided into two subclones. Partial sequence of the ends of the 2.1 kb subclone indicated that it contained a coding frame homologous to the *E. coil* COOH-terminal portion of the α subunit of glycyl-tRNA synthetase *(glyS)* and the majority of the β subunit of this gene (Webster et al., 1983, J. Biol. Chem. 258:10637). The predicted length of DNA needed to match the *E. coli* sequence was present; this clone was not examined further. --

Please delete the paragraph from lines 1-9 on page 42, and insert the following paragraph in place thereof:

-- *DNA Sequence of the LOS Locus.* A summary of the features found by sequencing the two clones is illustrated in Figure 2. Following the *glyS* gene were foundfive closely spaced open reading frames. The last frame has 46 bp downstream of the termination codon a sequence typical of a rho independent termination signal. Subsequently, there is an area of ca 100 bp that has striking homology to the ISI1106 neisserial insertion sequence (Knight et al., 1992, Molec. Microbiol. 6:1565). Further elucidation of the nature of this locus, presented below, showed the five open reading frames code for LOS glycosyl transferases and hence they have been named *lgtA- lgtrE.* --

Please delete the paragraph from lines 10-29 on page 42, and insert the following paragraph in place thereof:

-- Searches for internal homology within this locus indicates that the DNA coding for the first two genes *(lgtA, lgtB)* is repeated as the fourth and fifth genes *(lgtD, lgtE)* and that interposed is an additional open reading frame, *lgtC.* This is in keeping with the data obtained by Southern hybridization presented above, in which pR10PI probe containing the *lgtB* and a small portion of the *lgtC* gene hybridized with two *CIa*I fragments, but with only one *Bfa*I fragment (see positions of the *Bfa*I sites in the LOS locus in Figure 2). In more detail, 16 bp following the stop codon of the tRNA synthetase *(glyS)* is the beginning of a stem loop structure followed closely by a consensus ribosome binding site (rbs), and within 6 bp is a TTG believed to be the initiation codon of *lgtA.* 2871 bp downstream from the beginning of the stem loop (closely following the stop codon of *lgtC)* there is an almost perfect repeat of the stem loop structure, the rbs, and the TTG initiation codon of *lgtD,* with the downstream sequence strongly homologous for about 500 bp. The sequences then diverge to some extent. However, at the beginning of *lgtB* and *lgtE* the homology again becomes nearly perfect for ca 200 bases to then diverge toward the latter part of the orfs. The similarity of the homologous proteins is illustrated in Figures 3 and 4. These comparisons, demonstrate the near-perfect conservation of the primary structure in the N-terminal portions of the molecules with increasing divergence toward the COOH-termini of the proteins. --

In the Drawings:

Please amend Figure 2 by deleting Figure 2B. A marked-up copy and a clean copy of Figure 2 are provided herewith.

In the Claims:

Please amend claims 34-39 without prejudice, as set forth below. A "marked-up" copy of the claims indicating the changes is attached hereto.

34. (Amended) An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:8, or a functionally active fragment thereof.

35. (Amended) An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:3, or a functionally active fragment thereof.

36. (Amended) An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:4, or a functionally active fragment thereof.

37. (Amended) An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:5, or a functionally active fragment thereof.

38. (Amended) An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:6, or a functionally active fragment thereof.

39. (Amended) A composition comprising an isolated glycosyltransferase conjugated to a solid phase support, wherein the glycosyltransferase is selected from the group consisting of:

a) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:8, or a functionally active fragment thereof;

b) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:3, or a functionally active fragment thereof;

c) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:4, or a functionally active fragment thereof;

d) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:5, or a functionally active fragment thereof;

e) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:6, or a functionally active fragment thereof.

REMARKS

Claims 34-49 are pending in the current application. By way of the present Amendment, claims 34-39 are amended.

Claims 34-49 are pending in the application. Claims 1-26 and 28-31 were canceled by way of the first Preliminary Amendment, filed on December 3, 2001, with the instant application. Claims 27, 32, and 33 were cancelled and claims 34-49 were added by way of the second Preliminary Amendment, filed on April 12, 2002. Therefore, claims 34-49 are presently under consideration.

Claims 34-39 have been amended herein to more particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Support for these amendments is found throughout the specification as filed as more fully set forth below. Thus, no new matter has been added by way of these amendments.

Amendments to the Application to Correct Typographical Errors

Applicant has amended the specification herein merely to correct inadvertent typographical and editing errors, and to correctly identify the Sequence Identification numbers.

As indicated in the Preliminary Amendment filed along with the present application on December 3, 2001, the present application is related to U.S. application no. 08/878,360, filed June 18, 1997, now U.S. Patent 5,945,322, U.S. application no. 08/683,426, filed July 18, 1996, now U.S. Patent 5,705,367, and U.S. application no. 08/312,387, filed September 6, 1994, now U.S. Patent 5,545,553. The amendments to the specification described in detail herein below correspond to amendments made in each of

the above-referenced related patent applications for the purpose of correcting the same inadvertent typographical and editing errors, and accordingly, the present amendments merely serve to ensure that the present application is in conformance with the applications to which it claims priority.

Sequence Identification Amendments

Applicant has amended the specification herein merely to correct inadvertent typographical and editing errors regarding the Sequence Identification numbers, and to correctly identify the Sequence Identification numbers.

SEQ ID NOS:11 and 12 refer to LgtA and LgtD polypeptides, respectively, as set forth in Figure 3 as originally filed. SEQ ID NOS:11 and 12 *correspond* to LgtA and LgtD polypeptides as set forth in SEQ ID NOS:3 and 5, respectively, with the only exception being that the first amino acid is leucine rather than methionine. For example, though the lgtA TTG codon in the corresponding position in SEQ ID NO:1 ordinarily encodes leucine (SEQ ID NO:1 nucleotides 445-447), in *Neisseria*, the same TTG codon encodes methionine. Thus, the sequences of SEQ ID NOS:3 and 5 are correct. However, the amino acid sequences for LgtA and LgtD polypeptides in Figure 3 (SEQ ID NOS:11 and 12, respectively) include leucine as the first amino acid, such as would be found if the protein were expressed in a non-*Neisseria* expression system, such as those expression systems described in the specification at page 25, lines 1-12.

Amendments to the Drawings

Figure 2 has been amended to delete Figure 2B, which corresponds to the sequence listing submitted with the present application. Applicants submit herewith seven (7) sheets of cancelled drawings corresponding to Figure 2B, and eleven (11) sheets of amended formal drawings. The drawings have merely been amended to renumber the sheets of drawings as a result of the cancellation of Figure 2B, and accordingly, no new matter has been added by way of this amendment.

Amendments to Claims 34 and 39

Claims 34 and 39 have been amended to correctly refer to the Sequence Identification numbers, which have been amended in the above-described amendments to the specification to correct inadvertent typographical and editing errors.

The present application claims, in part, an invention comprising five (5) novel glycosyltransferases from *Neisseria*. Specifically, the present application claims lgtA, lgtB, lgtC, lgtD, and lgtE polypeptides.

Accordingly, the five glycosyltransferases of the present invention are the object of claims 34-39, as indicated by the recitation of the identifying term, "glycosyltransferase," followed by the recitation of a SEQ ID number for each one of the LgtA, LgtB, LgtC, LgtD, and LgtE glycosyltransferases. SEQ ID NO:3 correctly refers to lgtA, SEQ ID NO:4 correctly refers to lgtC, SEQ ID NO:5 correctly refers to lgtD, and SEQ ID NO:6 correctly refers to lgtE. However one of the five glycosyltransferases of the present invention, LgtB, was incorrectly referred to using the term "SEQ ID NO:2" in claims 34 and 39.

SEQ ID NO:2, as set forth in the Sequence Listing in the present application, is a polynucleotide sequence encoding a glycyl tRNA synthetase beta chain, and is not a *Neisseria* glycosyltransferase. SEQ ID NO:8 correctly refers to LgtB, a glycosyltransferase of the present invention. Therefore, the above-described amendments to the application serve in part to make proper reference to the LgtB glycosyltransferase (SEQ ID NO:8) of the present invention, and to eliminate any incorrect reference to glycyl tRNA synthetase (SEQ ID NO:2), which is not claimed as part of the present invention.

More particularly, claims 34 and 39 have been amended in part to correctly refer to the Sequence Identification numbers, which numbers have been amended by way of the above-described amendments to the specification to correct inadvertent typographical and editing errors. Specifically, the incorrect recitation of "SEQ ID NO:2" in claims 34 and 39 has been changed so that the claims now correctly recite "SEQ ID NO:8." Prior to the instant amendment, claims 34 and 39 incorrectly recited, "...glycosyltransferase comprising the amino acid sequence SEQ ID NO:2, or a functionally active fragment thereof." Amended claims 34 and 39 now properly recite

"SEQ ID NO:8," which is the LgtB glycosyltransferase of the present invention.

All of the above-detailed amendments to the application have merely been made to correct informalities with the present application. Accordingly, Applicant respectfully submits that no new matter has been added by way of the amendments. Support for all of the above-described amendments to the application can be found in the as-filed application, and specifically, in the Sequence Listing.

The Sequence Listing filed in connection with the present application sets forth that SEQ ID NO:3 is LgtA, SEQ ID NO:4 is LgtC, SEQ ID NO:5 is LgtD, SEQ ID NO:6 is LgtE, and that SEQ ID NO:8 is LgtB. Specifically, SEQ ID NO:1 sets forth a *Neisseria* polynucleotide sequence which also displays the amino acid translation of the coding regions of SEQ ID NO:1. The "Information for SEQ ID NO:1" set forth on pages 1 and 2 of the Sequence Listing of the present application defines SEQ ID NO:1 as encoding glycyl tRNA synthetase beta chain from nucleotides 1-381 of SEQ ID NO:1, encoding LgtA from nucleotides 445-1491 of SEQ ID NO:1, encoding LgtC from nucleotides 2342-3262 of SEQ ID NO:1, encoding LgtD from nucleotides 3322-4335 of SEQ ID NO:1, and encoding LgtE from nucleotides 4354-5196 of SEQ ID NO:1.

Each of the five proteins encoded by SEQ ID NO:1 – four glycosyltransferases and one glycyl tRNA synthetase – are set forth in SEQ ID NOS:2-6 in the Sequence Listing. SEQ ID NO:2 sets forth the amino acid sequence for the glycyl tRNA synthetase beta chain encoded by nucleotides 1-381 of SEQ ID NO:1, SEQ ID NO:3 sets forth the amino acid sequence for the LgtA glycosyltransferase encoded by nucleotides 445-1491 of SEQ ID NO:1, SEQ ID NO:4 sets forth the amino acid sequence for the LgtC glycosyltransferase encoded by nucleotides 2342-3262 of SEQ ID NO:1, SEQ ID NO:5 sets forth the amino acid sequence for the LgtD glycosyltransferase encoded by nucleotides 3322-4335 of SEQ ID NO:1, and SEQ ID NO:6 sets forth the amino acid sequence for the LgtE glycosyltransferase encoded by nucleotides 4354-5196 of SEQ ID NO:1.

Also in the Sequence Listing filed in connection with the present application, SEQ ID NO:7 sets forth a *Neisseria* polynucleotide sequence which also displays the amino acid translation of the coding regions of SEQ ID NO:7. The "Information for SEQ ID NO:7" set forth on page 14 of the Sequence Listing of the

present application defines SEQ ID NO:7 as encoding LgtB from nucleotides 1491-2330 of SEQ ID NO:7. SEQ ID NO:8 sets forth the amino acid sequence for the LgtB glycosyltransferase encoded by nucleotides 1491-2330 of SEQ ID NO:7.

Rejection under 35 U.S.C. § 101

The Examiner has rejected claims 34-49 under 35 U.S.C. § 101 because, in the Examiner's view, the claimed invention is non-patentable, as the claimed invention is directed to "a glycosyltransferase," which reads on "a product of nature." Applicants, while not necessarily agreeing with the Examiner's reasoning, in a good faith effort to expedite prosecution of this application, have amended claims 34-39, from which claims 40-49 depend, to recite "an isolated glycosyltransferase" instead of "a glycosyltransferase." This amendment introduces no new subject matter and is supported throughout the specification and claims as filed (e.g., specification at page 13 lines 5-10, and at page 19 line 21 through page 24 line 22). Applicants respectfully submit that claims 34-49, as amended, are indeed patentable and accordingly request withdrawal of the rejection.

Rejection under 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 34-49 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out and distinctly claim the subject matter that the applicant regards as the invention. Specifically, it is the Examiner's view that Applicant's do not clearly define the "functional activity" with respect to the claimed invention.

Applicants respectfully contend that "functional activity" of a glycosyltransferase of the invention is clearly defined throughout the specification as filed. For example, lines 10-15 on page 22 of the specification define a "functionally active fragment" as a glycosyltransferase fragment that is capable of mediating transfer of a sugar to an acceptor molecule. The definition is further clarified by contrast with functionally inactive fragments at lines 16-20 on page 22 of the specification, which fragments are not required for functional activity as described above.

Additionally, specific examples of the activity of functionally active

glycosyltransferases are given at page 7, lines 6-10. Further, lines 1-5 on page 21 describe how one skilled in the art can identify a functionally active fragment of a glycosyltransferase of the present invention, in particular by the ability of such a fragment to mediate transfer of a sugar to an acceptor molecule. Accordingly, Applicants submit that the term "functionally active fragment thereof" is not indefinite, and request that the rejection be reconsidered and withdrawn.

Rejection under 35 U.S.C. § 103(a)

The Examiner has rejected claim 39 under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,180,674 of Roth ("the '674 patent"). Specifically, it is the Examiner's view that the '674 patent teaches a method of immobilization of a glycosyltransferase to a solid support, and that one of skill in the art would have been sufficiently motivated to immobilize specific glycosyltransferases of the present invention in light of the '674 patent.

Prior to Applicant's rebuttal of the Examiner's rejection, Applicant wishes to point out that the SEQ ID numbers referred to hereinbelow are the correct, amended SEQ ID numbers for the five glycosyltransferases of the present invention, as described in greater detail above. Specifically, SEQ ID NOS:3-6 and 8 refer to glycosyltransferases lgtA, lgtC, lgtD, lgtE, and lgtB, respectively.

Applicants respectfully submit that the embodiment of the present invention set forth in claim 39 would **not** have been obvious to one of skill in the art, and that the '674 patent does not render claim 39 *prima facie* obvious under 35 U.S.C. § 103(a), for the following reasons.

The three-prong test which must be met for a reference or a combination of references to establish a *prima facie* case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references

when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

Not all of these criteria have been met here.

Assuming, *arguendo*, that one of skill in the art would have been motivated to attempt to immobilize a glycosyltransferase on a solid support, and with a reasonable expectation of success, the overriding fact remains that all of SEQ ID NOS:3-6 and 8, glycosyltransferases lgtA, lgtC, lgtD, lgtE, and lgtB, respectively, were **unknown before the time of filing of the present patent application**. None of the prior art cited by the examiner - neither the cited prior art reference (the '674 patent) nor the knowledge of one skilled in the relevant art prior to the time of filing of the present application – teaches or suggests **all** of the claim limitations. Particularly, because *Neisseria* glycosyltransferases lgtA, lgtC, lgtD, lgtE, and lgtB (SEQ ID NOS:3-6 and 8, respectively) were **unknown** before the time of filing of the present patent application, such limitations of instant claim 39 would not have been available to one of skill in the art to rely upon for teaching or suggesting the present invention in conjunction with the '674 patent. Thus, the combination of the prior art cited cannot render the present invention *prima facie* obvious under 35 U.S.C. 103(a).

In fact, the Examiner's rejection is based on improper hindsight reasoning. The sequences represented by SEQ ID NOs:3-6 and 8 in the present application are novel amino acid sequences disclosed for the first time in the present invention. Consequently, one of skill in the art would **only** be motivated to immobilize the glycosyltransferases represented by SEQ ID NOs:3-6 and 8 when armed with the disclosure of the present application. See MPEP 2145(X). Accordingly, Applicants respectfully submit that the rejection is improper and should be withdrawn.

Double Patenting Rejection

The Examiner has rejected claims 35-39, 41-44, and 46-49 under the judicially created doctrine of obviousness-type double patenting. Specifically, it is the Examiner's view that the above-mentioned claims are not patentably distinct from claims 1-5, 7-10, 12-16 and 18 of U.S. Patent No. 5,798,233 of Gotschlich ("the '233 patent").

Applicant understands that a timely filed Terminal Disclaimer in compliance with 37 CFR § 1.321(c) may be used to overcome such a non-statutory type of double patenting rejection. Accordingly, Applicant is filing a Terminal Disclaimer herewith to overcome the double patenting rejection of claims 35-39, 41-44, and 46-49.

Sequence Compliance

In the Office Action, the Examiner requested compliance with sequence rules. Specifically, the Examiner noted that the sequences depicted in Figures 3, 4 and 5 are not identified with a SEQ ID number.

Applicant has amended the Brief Descriptions of Figures 3, 4 and 5 to formally identify each of the amino acid sequences in Figures 3, 4 and 5 with a SEQ ID number. Specifically, Applicant has inserted the corresponding SEQ ID number following each recitation of the name of the amino acid sequence in the Brief Descriptions of Figures 3, 4 and 5. More particularly, Figure 3 illustrates an amino acid sequence comparison of LgtA and LgtD, SEQ ID NOS:11 and 12, respectively, Figure 4 illustrates an amino acid sequence comparison of LgtB and LgtE, SEQ ID NOS:8 and 6, respectively, and Figure 5 illustrates an amino acid sequence comparison between LgtC and rfaI, SEQ ID NOS:4 and 13, respectively.

Further, Applicant has submitted an amended copy of the Sequence Listing, which amended copy is identical to the Sequence Listing originally filed with the present application except for the addition of SEQ ID NO:13, which is the rfaI sequence found in Figures 5A and 5B. Applicant respectfully submits that the amino acid sequence of SEQ ID NO:13 is identical to the rfaI amino acid sequence set forth in Figures 5A and 5B. Accordingly, no new matter has been added to the application by way of this amendment.

Summary

The amendments made herein are supported in the as-filed specification, and as such, no new matter has been added by way of the present amendment. Applicant respectfully submits that each and every rejection or objection set forth by the Examiner has either been overcome or is now inapplicable, and that the instant application is in full condition for allowance. Favorable examination of the claims on the merits is respectfully requested.

Respectfully submitted,
EMIL C. GOTSCHLICH

MARCH 10, 2003
(Date)

By: _____

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KD/TMS/QDN

Enclosures (Sequence Listing, CRF of Sequence Listing, Statement to Support Sequence Listing, Drawing Transmittal Sheet, Seven Sheets of Cancelled Drawings, Eleven Sheets of Amended Formal Drawings, Terminal Disclaimer, Terminal Disclaimer Transmittal Sheet)

Marked-up version of the specification to show changes made

Please delete the paragraph from page 4 line 15 to page 5 line 2, and insert the following paragraph in place thereof:

-- Little information on the genetics of LOS synthesis ~~of~~ in Neisseria is available. A major advance has been the creation (Dudas and Apicella, 1988, Infect. Immun. 56:499) and biochemical characterization (John et al., 1991, J. Biol. Chem. 266:19303) of five pyocin mutants of gonococcal strain 1291, dubbed 1291a-e. Immunological and biochemical data have shown that 1291a, 1291c, 1291d and 1291e produce LOS with sequential shortening of the lacto-N-neotetraose chain, with mutant 1291e lacking the glucose substitution on the heptose. Mutant 1291b synthesizes the alternative LOS structure Galα1 $\rightarrow$ 4Galβ1 $\rightarrow$ 4Glc (see Figure 1). Only the genetic basis of the 1291e mutant is now defined. It is a mutation of phosphoglucomutase (pgm), which precludes the synthesis of UDP-glucose, and hence the addition of the first residue of the lacto-N-neotetraose unit (Zhou et al., 1994, J. Biol. Chem. 269:11162; Sandlin and Stein, 1994, J. Bacteriol. 176:2930). It also has been shown that gale mutants of meningococcus or gonococcus produce truncated LOS in keeping with the inability to synthesize UDP-galactose (Robertson et al., 1993, Molec. Microbiol. 8:891; Jennings et al., 1993, Molec. Microbiol. 10:361). --

Please delete the paragraph at lines 13-23 on page 6 and insert the following paragraph in place thereof:

-- The present invention is directed to nucleic acids encoding glycosyltransferases, the proteins encoded thereby, and to methods for synthesizing oligosaccharides using the glycosyltransferases of the invention. Accordingly, in one aspect, the invention is directed to a purified nucleic acid that is hybridizable under moderately stringent conditions to a nucleic acid corresponding to the LOS locus of Neisseria, e.g., a nucleic acid having a nucleotide sequence corresponding to or complementary to the nucleotide sequence shown in ~~Figure 2~~ (SEQ ID NO:1). Preferably, the nucleic acid of the invention is hybridizable to a portion of the coding sequence for a gene of the LOS locus, i.e., a portion of the nucleotide sequence shown in

~~Figure 2~~ (SEQ ID NO:1) that encodes a functionally active glycosyltransferase. --

Please delete the paragraph on page 6 line 24 to page 7 line 5 and insert the following paragraph in place thereof:

-- In specific embodiments, the invention relates to a nucleic acid that has a nucleotide sequence corresponding to or complementary to a portion of the nucleotide sequence shown in ~~Figure 2~~ (SEQ ID NO:1) that encodes a functionally active glycosyltransferase. In a further aspect, the nucleic acid encodes a functionally active glycosyltransferase. In a specific embodiment, the invention is directed to a nucleic acid that has a nucleotide sequence corresponding to or complementary to the nucleotide sequence shown in ~~Figure 2~~ (SEQ ID NO: 1). --

Please delete the paragraph at lines 15-17 on page 7 and insert the following paragraph in place thereof:

B1

-- In specific embodiments, exemplified herein, the nucleic acid encodes a glycosyltransferase having an amino acid sequence of ~~SEQ ID NO:2;~~ SEQ ID NO:3 or SEQ ID NO:11, SEQ ID NO:4, SEQ ID NO:5~~,~~ or SEQ ID NO:12, SEQ ID NO:6, or SEQ ID NO:8. --

Please delete the paragraph at lines 1-13 on page 8 and insert the following paragraph in place thereof:

B2

-- In a primary aspect, the invention is directed to glycosyltransferase having an amino acid sequence of ~~SEQ ID NO:2,~~ SEQ ID NO:3 or SEQ ID NO:11, SEQ ID NO:4, SEQ ID NO:5~~, or~~ or SEQ ID NO:12, SEQ ID NO:6, or SEQ ID NO:8 or a functionally active fragment thereof. The invention further contemplates a composition comprising a glycosyltransferase conjugated to a solid phase support, wherein the glycosyltransferase is selected from the group consisting of a glycosyltransferase having an amino acid sequence of ~~SEQ ID NO:2~~ SEQ ID NO:3 or SEQ ID NO:11, or a functionally active fragment thereof; a glycosyltransferase having an amino acid sequence of SEQ ID ~~NO:3~~ NO:8, or a functionally active fragment thereof; a glycosyltransferase having an amino acid sequence of SEQ ID NO:4, or a functionally

active fragment thereof; and a glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12, or a functionally active fragment thereof; and a glycosyltransferase having an amino acid sequence of SEQ ID NO:6, or a functionally active fragment thereof. --

Please delete the paragraph from page 8 line 14 to page 9 line 3, and insert the following paragraph in place thereof:

-- Having provided novel glycosyltransferases, and genes encoding the same, the invention accordingly further provides methods for preparing oligosaccharides, *e.g.*, two or more saccharides. In specific embodiments, the invention relates to a method for adding GalNAc or GlcNAcβ1→3 to Gal, comprising contacting a reaction mixture comprising an activated GalNAc or GlcNAc to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID ~~NO:2~~ NO:3 or SEQ ID NO:11; a method for adding Galβ1→4 to GlcNAc or Glc, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a GlcNAc or Glc residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID ~~NO:3~~ NO:8; a method for adding Gal αl→4 to Gal, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:4; a method for adding GalNAc or GlcNAc β1→3 to Gal, comprising contacting a reaction mixture comprising an activated GalNAc or GlcNAc to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12; and a method for adding Gal β1→4 to GlcNAc or Glc, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a GlcNAc or Glc residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:6. --

Please delete the paragraph from lines 3-12 on page 11, and insert the following paragraph in place thereof:

-- Figure 2: ~~(A)~~ Genetic map of the LOS locus based on the DNA sequence. Sequence information bp 1-2725 was obtained from plasmid pPstCla, bp 2725-5859 from plasmid p3400 (see materials and methods). IS refers to an area of the sequence that has homology to a previously reported neisserial insertion sequence IS1106 (Knight et al., 1992, Molec. Microbiol. 6:1565). The positions of the reading frames of *lgtA-E* are indicated. Three tracts of poly-G were found in *lgtA* (17 bp), *lgtC* (10 bp) and *lgtD* (11 bp) and are indicated by vertical black bars. ~~(B) Amino acid sequences of LgtA (SEQ ID NO:2), LgtB (SEQ ID NO:3), LgtC (SEQ ID NO:4), LgtD (SEQ ID NO:5), and LgtE (SEQ ID NO:6), and the nucleotide sequence of the lgt locus (SEQ ID NO:1).~~ --

Please delete the paragraph from lines 13-18 on page 11, and insert the following paragraph in place thereof:

-- Figure 3 (A and B): Homology of the protein products of *lgtA* (SEQ ID NO:11) and *lgtD* (SEQ ID NO:12). The primary structure of two proteins is very similar, particularly in the first half of the sequences. The glycine residues starting at position 86 reflect the coding of the poly-G regions in the respective genes. The Bestfit program of the GCG package was used and the symbols |, :, . represent degrees of similarity based on the Dayhoff PAM-250 matrix. --

Please delete the paragraph from lines 19-23 on page 11, and insert the following paragraph in place thereof:

-- Figure 4 (A and B): Homology of the protein products of *lgtB* (SEQ ID NO:8) and *lgtE* (SEQ ID NO:6). The primary 20 structure of two proteins is very similar, particularly in the first half of the sequences. These sequences also have significant homology to *lex-1* (Cope et al., i99i, Molec. Microbiol. 5:i113) or *lic2A* (High et al., i993, Molec. Microbiol. 9:1275) genes of *Haemophilus influenzae*. For meaning of symbols see Figure 3. --

Please delete the paragraph from page 11 line 24 to page 12 line 2, and insert the following paragraph in place thereof:

-- Figure 5 (A and B): Homology of the protein products of *rfaI* (SEQ ID NO:13) and *lgtC* (SEQ ID NO:4). The *E. coli rfaI* and *rfaJ* genes are very closely related. They serve as glucosyl transferases of two glucose residues in the LPS core region (Pradel et al., 1992, J. Bacteriol. 174:4736). The glycines at position 54-56 in *lgtC* are encoded by the poly-G tract. For meaning of symbols see Figure 3. --

Please delete the paragraph from lines 11-29 on page 30, and insert the following paragraph in place thereof:

-- Accordingly, a method for preparing an oligosaccharide having the structure GalNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc *(i.e.*, ganglioside) comprises sequentially performing the steps of:

d. contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO: 6, or a functionally active fragment thereof;

e. contacting a reaction mixture comprising an activated GlcNAc to the acceptor moiety comprising a Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID ~~NO:2~~ NO:3 or SEQ ID NO:11, or a functionally active fragment thereof;

f. contacting a reaction mixture comprising an activated Gal to the acceptor moiety comprising a GlcNAcβ1→3Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid of SEQ ID ~~NO:3~~ NO:8; and

g. contacting a reaction mixture comprising an activated GalNAc to the acceptor moiety comprising a Galβ1→4GlcNAcβ1→3Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12, or a functionally active fragment thereof. --

Please delete the paragraph from lines 1-14 on page 31, and insert the following paragraph in place thereof:

-- Similarly, a method for preparing an oligosaccharide having the structure Galβ1→4GlcNAcβ1→3Ga1β1→4Glc *(i.e.,* lacto-N-neotetraose) comprises sequentially performing the steps of:

h. contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO: 6, or a functionally active fragment thereof;

i. contacting a reaction mixture comprising an activated GlcNAc to the acceptor moiety comprising a Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID ~~NO:2~~ NO:3 or SEQ ID NO:11, or a functionally active fragment thereof; and

j. contacting a reaction mixture comprising an activated Gal to the acceptor moiety comprising a G1cNAcβ1→3Ga1β1→4Glc residue in the presence of a glycosyltransferase having an amino acid of SEQ ID ~~NO:3~~ NO:8. --

Please delete the paragraph from lines 10-26 on page 38, and insert the following paragraph in place thereof:

-- A gene bank of *Neisseria gonorrhoeae* strain F62 genomic DNA was constructed by ligating ca 20 kb fragments obtained by incomplete digestion with *Sau3A* into *Bam*HI/*Eco*RI digested λ2001 (Karn et al., 1984, Gene 32:217). The phage library was screened by hybridization with ~~random-prime-labeled~~random-primer-labeled plasmid pR10PI, and 5 clones were isolated by plaque purification. The phage from these clones were purified by sedimentation followed by flotation on CsCl (Davis et al., 1980, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), and the DNA was isolated. From one of these clones, two *Cla*I fragments of 4.9 and 3.4 kb were isolated by gel electrophoresis and recovery with Geneclean II (BIO 101 Inc., La Jolla, CA). These were ligated into *Cla*I cut pBluescript II SK- from Stratagene (La Jolla, CA) and called p4900 and p3400 respectively. p4900 contained a *Pst*I site in the insert and was subdivided into two clones containing inserts of 2.1 and 2.8 kb. The clone containing the 2.8 kb insert was called pPstCla. The inserts in p3400 and pPstCla were sequenced by

the chain termination method (Sanger et al., 1977, Proc. Natl. Acad Sd. USA 74:5463) using Sequenase II, (United States Biochemical Co., Cleveland, OH). All of the sequence presented in ~~Figure 2~~SEQ ID NO:1 -was completed in both directions. --

Please delete the paragraph from line 13 page 39 to line 3 page 40, and insert the following paragraph in place thereof:

-- Transformation of piliated *Neisseria gonorrhoeae* strain F62 was performed with plasmids isolated from *E. coil* (Klugman et al., 1989, Infect. Immun. 57:2066) and the transformants selected on GC agar (Swanson, 1978, Infect. Immun. 19:320) containing 2 μg/ml erythromycin. The fidelity of the genomic alteration of each of the gonococcal transformants was verified by sequencing the upstream and downstream junctions of the *ermC'* gene in their genomic DNA using a PCR technique. Two 5' biotinylated primers, GCCGAGAAAACTATTGGTGGA (SEQ. ID. ~~NO:7~~ NO:9) and AAAACATGCAGGAATTGACGAT) (SEQ. ID. ~~NO:8~~ NO:10), were synthesized; these were based on the *ermC'* sequence near its upstream and its downstream end respectively. The primers were designed such that their 3' ends pointed outward from the *ermC'* gene. Each of these primers was used together with a suitable primer matching the sequence of the LOS locus near the putative insertion. PCR was performed according the instructions supplied with the GeneAmp PCR Reagent Kit from Perkin Elmer (Branchburg, NJ) using 25 cycles. In all instances the expected size product was obtained. The DNA sequence of these products was determined by purifying the PCR product on magnetic streptavidin beads from Dynal, Inc. (Lake Success, NY) and sequencing with the Sequenase II kit according to a protocol provided by Dynal, Inc., based on the method developed by Hultman et al (Hultman et al., 1989, Nucleic Acids Res. 17:4937). The sequences were analyzed by computer programs in the GCG package of Genetics Computer Group, Inc. (Madison, WI). --

Please delete the paragraph from lines 15-29 on page 41, and insert the following paragraph in place thereof:

-- A λ2001 bank of *Neisseria gonorrhoeae* strain F62 DNA was screened by hybridization with pR10PI and 5 clones were isolated. One of these clones, when

digested with either *Cla*I or *Bfa*I and examined by Southern hybridization using pR10PI as the probe, gave rise to a pattern identical to that seen with genomic DNA. The appropriate *Cla*I fragments of this λ2001 clone were isolated and cloned into the *Cla*I site of pBluescript II SK-. The entire sequence of the 3400 *Cla*I fragment was determined. Mapping of the clone containing the 4900 bp *Cla*I fragment indicated that there was a single *Pst*I site in the clone about 2.8 kb from one side, allowing the clone to be divided into two subclones. Partial sequence of the ends of the 2.1 kb subclone indicated that it contained a coding frame homologous to the *E. coil* COOH-terminal portion of the α subunit of glycyl-tRNA synthetase *(glyS)* and the majority of the β subunit of this gene (Webster et al., 1983, J. Biol. Chem. ~~258:10637~~ 258:10637). The predicted length of DNA needed to match the *E. coli* sequence was present; this clone was not examined further. --

Please delete the paragraph from lines 1-9 on page 42, and insert the following paragraph in place thereof:

-- *DNA Sequence of the LOS Locus.* A summary of the features found by sequencing the two clones is illustrated in Figure 2. Following the *glyS* gene ~~If found~~were found-five closely spaced open reading frames. The last frame has 46 bp downstream of the termination codon a sequence typical of a rho independent termination signal. Subsequently, there is an area of ca 100 bp that has striking homology to the ISI1106 neisserial insertion sequence (Knight et al., 1992, Molec. Microbiol. 6:1565). Further elucidation of the nature of this locus, presented below, showed the five open reading frames code for LOS glycosyl transferases and hence they have been named *lgtA- lgtrE.* --

Please delete the paragraph from lines 10-29 on page 42, and insert the following paragraph in place thereof:

-- Searches for internal homology within this locus indicates that the DNA coding for the first two genes *(lgtA, lgtB)* is repeated as the fourth and fifth genes *(lgtD, lgtE)* and that interposed is an additional open reading frame, *lgtC.* This is in keeping with the data obtained by Southern hybridization presented above, in which

pR10PI probe containing the *lgtB* and a small portion of the *lgtC* gene hybridized with two *CIa*I fragments, but with only one *Bfa*I fragment (see positions of the *Bfa*I sites in the LOS locus in Figure 2). In more detail, 16 bp following the stop codon of the tRNA synthetase *(glyS)* is the beginning of a stem loop structure followed closely by a consensus ribosome binding site (rbs), and within 6 bp is a TTG believed to be the initiation codon of *lgtA*. 2871 bp downstream from the beginning of the stem loop (closely following the stop codon of *lgtC)* there is an almost perfect repeat of the stem loop structure, the ~~rbs~~rbs, and the TTG initiation codon of ~~*lgtD*~~*lgtD*, with the downstream sequence strongly homologous for about 500 bp. The sequences then diverge to some extent. However, at the beginning of *lgtB* and *lgtE* the homology again becomes nearly perfect for ca 200 bases to then diverge toward the latter part of the orfs. The similarity of the homologous proteins is illustrated in Figures 3 and 4. These comparisons, demonstrate the near-perfect conservation of the primary structure in the N-terminal portions of the molecules with increasing divergence toward the COOH-termini of the proteins. --

Marked-up version of the claims to show changes made

Please amend claims 34-39 without prejudice, as set forth below.

34. (Amended) ~~A~~ An isolated glycosyltransferase comprising the amino acid sequence ~~SEQ ID NO:2~~SEQ ID NO:8, or a functionally active fragment thereof.

35. (Amended) ~~A~~ An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:3, or a functionally active fragment thereof.

36. (Amended) ~~A~~ An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:4, or a functionally active fragment thereof.

37. (Amended) ~~A~~ An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:5, or a functionally active fragment thereof.

38. (Amended) ~~A~~ An isolated glycosyltransferase comprising the amino acid sequence SEQ ID NO:6, or a functionally active fragment thereof.

39. (Amended) A composition comprising ~~a~~ an isolated glycosyltransferase conjugated to a solid phase support, wherein the glycosyltransferase is selected from the group consisting of:

a) a glycosyltransferase comprising the amino acid sequence ~~SEQ ID NO:2~~SEQ ID NO:8, or a functionally active fragment thereof;

b) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:3, or a functionally active fragment thereof;

c) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:4, or a functionally active fragment thereof;

d) a glycosyltransferase comprising the amino acid sequence SEQ ID NO:5, or a functionally active fragment thereof;

e) a glycosyltransferase comprising the amino acid sequence SEQ ID

NO:6, or a functionally active fragment thereof.

EXHIBIT C

U.S. Patent no. 5,945,322

Submitted in conjunction with the Amendment on (Date:) _____
responsive to the Office Action dated June 2, 2003 (Paper No. 10), sent in connection with U.S. Patent Application Number 10/007,267